

Steric constraints in model proteins

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A simple lattice model for proteins that allows for distinct sizes of the amino acids is presented. The model is found to lead to a significant number of conformations that are the unique ground state of one or more sequences or encodable. Furthermore, several of the encodable structures are highly designable and are the non-degenerate ground state of several sequences. Even though the native state conformations are typically compact, not all compact conformations are encodable. The incorporation of the hydrophobic and polar nature of amino acids further enhances the attractive features of the model.

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Protein folding remains a major unsolved problem in molecular biology. The successful design of proteins and enzymes with desired functionality and native state structure requires a knowledge of the rules underlying protein architecture¹. Simple exact models have proved to be invaluable in deducing the general principles of protein structure and stability²⁻⁴. Such models provide a coarse-grained description of proteins and have provided crucial insights on the role of inter-amino acid interactions (and in particular the hydrophobicity³ of some residues) in determining the secondary and tertiary structures of proteins. An even more fundamental structural principle pertains to steric constraints related to the diversity of residue sizes, the prohibition of overlaps of atoms and a close packing of the residues leading to small cavity volumes⁵. The principal theme of this paper is the introduction of a simple coarse-grained lattice model of a protein that consists of a sequence of amino acids configured in a self-avoiding conformation and which takes into account these steric constraints.

In its simplest form, the new model (that we will denote as the *LS* model) has two types of amino acids denoted by *L* (large) and *S* (small). (Further refinements taking into account a range of residue sizes is straightforward in principle but not essential to capture the role of steric constraints in determining protein architecture.) In terms of their interactions, the model is merely that of a homopolymer, a polymer made up of identical units, with an attractive energy proportional to the number of contacts between amino acids. Two amino acids are said to be in contact when they sit on adjoining sites but are yet not next to each other in sequence. Thus the lowest energy conformation of the sequence is one that has the largest possible number of contacts allowed by the steric interactions. Furthermore, if a particular sequence is able to be configured in a maximally compact conformation (which has the largest possible number of contacts and therefore is one of lowest energy), then any other degen-

erate ground state must also necessarily be maximally compact.

The consideration of steric constraints arises from postulating that in order to accommodate the large size of an *L* amino acid, at least one of the sites next to it must be kept vacant – no such constraint is imposed on the *S* amino acid. The steric constraint on the *L*-type amino acids immediately rules out the possibility of any maximally compact conformation in which *L* amino acids sit in the interior. Thus any allowed maximally compact conformation (which as stated above has an energy that cannot be improved upon) will have *S*-type amino acids in the interior and either *S* or *L*-type amino acids at the surface. We have carried out extensive tests of the *LS* model on a two dimensional square lattice and have found that it has many of the desirable attributes for modelling proteins:

1. the lowest energy states are typically compact;
2. only a tiny fraction of sequences admit a unique ground state (to ensure specificity);
3. there exist a significant number of encodable structures, i.e. structures which are the unique ground state of one or more sequences;
4. some of the encodable structures have a high degree of encodability and are thus highly designable, i.e. they are the non-degenerate ground state of several sequences.

Perhaps the simplest existing lattice model that satisfy these criteria is the *HP* model of Lau and Dill³ which also considers two kinds of amino acids denoted by *H* (hydrophobic) and *P* (polar). In the *HP* model, which has been studied widely, the protein collapse is driven by hydrophobic interactions between the *H* amino acids and the solvent. After integrating the solvent degrees of freedom, a more attractive effective *H* – *H* interaction

compared to the effective $P - P$ and $H - P$ interactions results. Thus, in its native state conformation, which is typically compact, the interior residues are usually hydrophobic and are thus shielded from the solvent. As a benchmark for our results, we will often refer to comparable results in the HP model with an attractive $H - H$ interaction and zero $H - P$ and $P - P$ interactions.

The LS model may be formally described by the Hamiltonian:

$$\mathcal{H} = - \sum_i [z_i(\Gamma)] \cdot A[z(\sigma_i) - z_i(\Gamma)] \quad (1)$$

where $\sigma_i \in \{L, S\}$, $z(\sigma_i)$ is the number of bonds belonging to residue i not used for chain connectivity and on a square lattice:

$$z(\sigma_i) = \begin{cases} 1 & \text{for } L \text{ residues inside the chain,} \\ 2 & \text{for } S \text{ residues inside the chain,} \\ 2 & \text{for } L \text{ residues at chain ends,} \\ 3 & \text{for } S \text{ residues at chain ends.} \end{cases} \quad (2)$$

$z_i(\Gamma)$ is the number of contacts of the i th residue in a conformation Γ and $A(x)$ is defined by

$$A(x) = \begin{cases} 1 & \text{if } x \geq 0, \\ -\infty & \text{otherwise.} \end{cases} \quad (3)$$

The function $A(x)$ is used to enforce residue incompressibility. In fact, when mounting a sequence on a structure, it may happen that a L residue is surrounded by four occupied sites. In this case $A[z(\sigma_i) - z_i(\Gamma)]$ diverges, thus assigning a $+\infty$ energy penalty to this forbidden situation. The model may be generalized in a straightforward way to higher dimensions and variations of sizes and the nature of steric constraints. The latter may be softened by allowing for a variety of possibilities but with an associated cost, for example by modifying the definition of $A(x)$.

We now describe the results of our exact enumeration studies on two-dimensional square lattices. Chan and Dill³ have shown that two dimensional models more faithfully capture the correct physically important surface-interior ratios of proteins than the corresponding three dimensional counterparts. They point out that in order to reproduce the correct ratio for a molecule of the size of myoglobin requires only 16-20 monomers in two dimensions as opposed to 154 in three dimensions. The latter case is clearly beyond the scope of exact enumeration. A more feasible size in three dimensions would consist of 27 monomers, but unfortunately has just one interior residue in a maximally compact conformation.

The core of the computational approach is made up of two backtracking procedures through which one generates the complete set of sequences and inequivalent structures of a given length – one exploits lattice symmetries to get rid of redundant structures. Following standard practice, we will assume that head to tail inversions is not an allowed symmetry operation and that we

are dealing with oriented walks. We have carried out a complete enumeration of all sequences of length 16 and all self-avoiding conformations for both the LS and HP models. Altogether there are $2^{16} = 65536$ different sequences and 802075 distinct oriented walks (69 of which are maximally compact and fill a 4x4 square). We laid out each sequence on each of the 802075 structures and determined its ground states. We kept track of both the sequences which happened to have a unique ground state and also its native conformation. For the LS model, the total number of sequences with a unique ground state was 7555, while the number of distinct encodable structures was 117. For each of these structures we calculated its encodability score, i.e., the number of sequences that admit it as the unique ground state. A summary of our results is presented in Table I.

It is striking that the most encodable structures for the LS model are maximally compact (Figure 1). Indeed there are a grand total of 7202 sequences (out of 7555) that admit a maximally compact structure as their unique native states. It is also important to note that not all maximally compact structures are encodable (thus preserving specificity). In fact, only 33 out of the 69 maximally compact structures are encodable. The encodability scores of these compact structures range from 60 up to 519. The HP model, on the other hand, admits 456 encodable structures and the highest encodability score is 26. Only 20 of these structures turn out to be maximally compact.

We have also carried out exact enumeration studies on chains of length 16 considering only the 69 maximally compact structures as target ground states. For the HP model, due to the absence of significant competing structures which are non-compact, all 69 conformations are recognized as encodable, with an associated loss of specificity. On the contrary the encodable maximally compact structures of the LS model are not affected. This property can be rigorously established by geometrical arguments, as well as the fact that, if a sequence admits a unique compact structure as a ground state, then it cannot be mounted on any other compact conformation without violating steric constraints. This implies an enhanced thermodynamic stability of encodable compact structures with respect to the HP model. In fact, the average energy gap of encodable structures measured on the compact ensemble is infinite for the LS model whereas it is finite for the HP case⁴.

We now turn to the case of sequences of length 25. The number of distinct structures of length 25 is too big to allow an exhaustive search for each of the 2^{25} sequences. However, because it appears that the most significant structures are the maximally compact ones, we simplified the task by considering only such structures as candidate ground states for each of the 2^{25} sequences. For the LS model, the number of sequences of length 25 that admit a unique ground state on one of the 1081 compact structures is 1340155. The number of encodable compact structures is 589 (Table I and Figure 2). It is

important to note that these numbers are rigorous lower bounds to the number that would be obtained, were one to consider the non-compact conformations as well. This follows from the observation that a maximally compact conformation has a lower energy than any non-compact conformation for the *LS* model.

The case of chains of 36 beads is interesting because they are sufficiently long to reveal the presence or absence of geometrical regularity in highly encodable structures. Again, to reduce the numerical task to manageable proportions, we will consider only the maximally compact structures that fit in a 6x6 square. There are altogether 57337 inequivalent compact structures. It is not numerically feasible to mount each of the 2^{36} *LS* sequences on the whole set of compact structures to determine whether they have a unique ground state. We therefore chose to explore a tiny portion of the sequence space by means of random sampling. By using a good random number generator⁶, we generated 12996000 random *LS* sequences. We found that 16611 of the compact structures were encodable with the encodability scores ranging between 1 and 64. Figure 3 shows pictures of the inequivalent structures with the top encodability scores and show motifs of emergent secondary structure. Interestingly, the most encodable structure is the same as the most designable structure found by Li *et al.*⁴ within the *HP* framework (for parameters $E_{HH} = -2.3$, $E_{HP} = -1.0$, $E_{PP} = 0.0$).

From the results presented so far, it appears that the *LS* model captures encodability and specificity better than the *HP* model suggesting that steric constraints could be as important as hydrophobic/polar interactions in determining protein architecture. An interesting avenue for further exploration would be a study of the kinetics of folding of the *LS* model and comparisons with the *HP* model. Another intriguing possibility is to combine the two classes (*LS* and *HP*) with the introduction of four species of residues: Large-Hydrophobic (*LH*), Large-Polar (*LP*), Small-Hydrophobic (*SH*) and Small-Polar (*SP*). Would one end up with a larger number of encodable structures or higher encodability scores? Would this cure the well-known defect of the pure *HP* model, namely that *HP* sequences have, on average, a huge ground-state degeneracy?

An exact statement that one may make is that if one considers *LS* sequences that have a unique maximally compact ground state, then on introducing an additional *H* or *P* character, the same ground state will be retained unless the new ground state of the *LSHP* model is no longer maximally compact. In order to address this issue quantitatively, we have carried out an exact enumeration of the 4^{12} sequences of length 12 on a square lattice. Our results (Table I) show that the *HP* diversity stabilizes new encodable structures (though **not** just compact ones!). Also note that there are many ways of choosing how to “fine-grain” the original *LS* sequence into a *LSHP* one (or equivalently many ways of “coloring” the *LS* residues in order to assign them a hydrophobic/polar

index). These observations are consistent with the idea that the introduction of a moderate diversity of the complementary kind may, indeed, improve the “good features” of the pure *LS* or *HP* models by enhancing the encodability of designable compact structures. It is also expected, on general grounds, that the increased amino acid diversity will cause sequences to have, on average, a smaller ground state degeneracy than in the pure *HP* lattice model.

Our studies show that the most encodable structures are indeed compact and that the smaller residues are more easily accommodated within the core of the protein. In order to minimize the energy of the native state of the protein, hydrophobic amino acids tend to be buried within the core (in order to avoid the solvent). Another way of enhancing the thermodynamic stability of the native state is by increasing the energies of the sequence in competing conformations⁷ and may result in polar amino acids being found in the core of the native state. The considerations presented here suggest that the smaller polar amino acids (Thr, Ser) ought to have a larger propensity for being buried than the larger ones (Lys, Glu and Arg) and this is indeed observed in studies of natural proteins⁸.

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Model	N	ES	CES	MDS
LS	12	15	15	24
HP	12	25	5	14
LSHP	12	232	31	$4 \cdot 10^5$
LS	16	117	33	519
HP	16	456	20	26
LS	25	—	589	12777

TABLE I. The chain length N , the number of Encodable Structures (ES), the number of Compact Encodable Structures (CES) and the Maximum Designability Score (MDS) on a square lattice. For $N = 25$, only maximally compact conformations were considered.

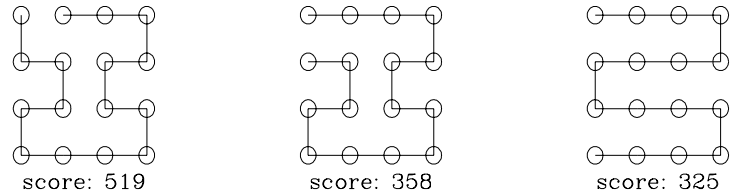


FIG. 1. The most encodable compact structures not related by any symmetry operation (with their designability score) for the *LS* model in $d = 2$ with $N = 16$ monomers. The data are obtained with an exact enumeration of all the sequences and all the conformations.

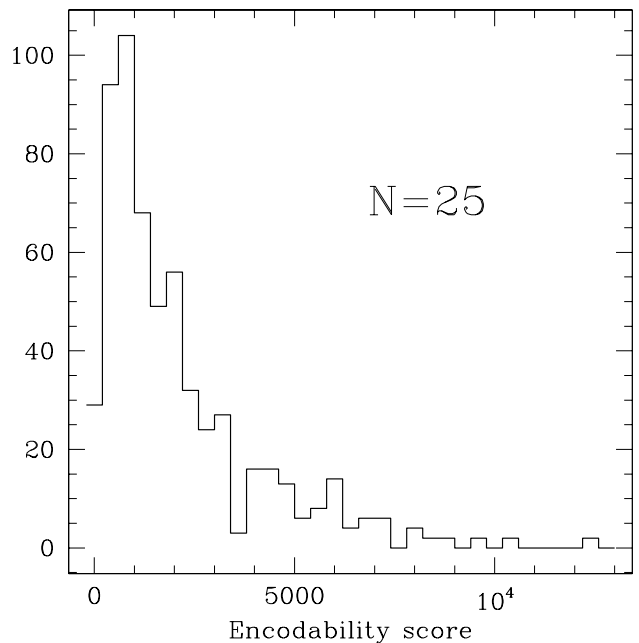


FIG. 2. Histogram of number of compact structures with a given encodability score for the LS model with $N = 25$.

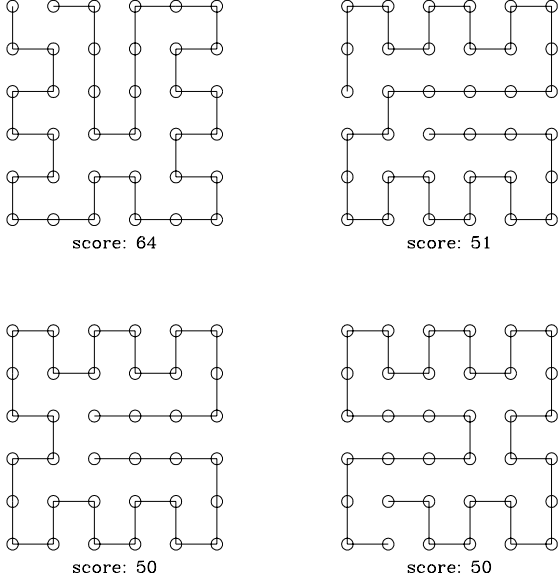


FIG. 3. The most encodable compact structures (with their score) for the LS model with $N = 36$. The results are obtained with a random sampling of the space of the sequences and a complete enumeration of all the compact structures.